Modelling the feasibility of intramolecular dehydrodiferulate formation in grass walls†‡

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Abstract: Molecular modelling is a useful tool for predicting and visualising molecular interactions, eg providing a means of predicting the feasibility of covalent bond formation between macromolecules. Molecular modelling was used to evaluate the feasibility of intramolecular diferulate formation. Two ferulates were positioned at various locations along the backbone of an arabinoxylan (16 xylose residues) and the optimised structure generated using MM2 parameters. For ferulates separated by several xylose residues, diferulates could only form if the xylan backbone relaxed allowing chain folding to bring the two ferulates within spatial proximity for bonding. In positions that would allow overlap of ferulates, one or both of the ferulates would have to rotate along the xylan backbone for radical coupling. In both cases high-energy barriers prevented the complete rotation to allow bond formation. It therefore seems unlikely that intramolecular dehydrodiferulates form readily within grass cell walls. When two xylose residues separate two arabinose moieties containing ferulate units it is feasible for rotation to a position allowing the formation of the 5–5 linked diferulate with no relaxation of the backbone. This is the only diferulate that can form without bond strain when the ferulates are positioned three xylose residues apart on the same xylan backbone. This suggests restricted positioning of ferulates for 5–5 coupling.

Keywords: cell walls; ferulates; diferulate formation; molecular modelling

INTRODUCTION

The structural and functional role of plant walls is regulated by complex interactions among wall components that involve covalent, ionic and hydrogen bonding.[1–5] Recently it was shown that grasses contain the full range of dehydrodiferulates predicted by radical coupling reactions, suggesting a greater prominence of cross-linking among wall polysaccharides then was previously recognised.[6,7] A high concentration of dehydrodiferulates implies extensive cross-linking of arabinoxylan thus changing the physical characteristics of the wall matrix. Arabinoxylan cross-linking, however, would not be as extensive if some dehydrodiferulates arose from intramolecular instead of intermolecular coupling. To test the feasibility of such intramolecular bond formation we utilised a molecular modelling program to visualise diferulate formation and predict optimum molecular structures of diferulates attached to a single arabinoxylan backbone.

METHODS

Molecular modelling experiments were carried out using the modelling program CAChe (Oxford Molecular Group). Arabinoxylan models were constructed to contain a backbone of 16 xylose residues. Pairs of feruloylated arabinofuranosyl units were added to the xylan backbone at various xylose units starting with the fourth unit from the reducing end. Arabinosyl residues were always coupled to the C3 hydroxyl of the xylose unit selected for attachment. The ferulate on the fourth xylose was held constant while the second ferulate was positioned at increasing distances along the xylan backbone starting with the adjacent xylose and increasing by one xylose residue for each set of coupling experiments (see Scheme 1). Molecular structures were optimised for low energy conformations using MM2 parameters. All optimal structures were moved from the low energy position and subjected to MM2 optimisation at least two additional times to ensure the lowest

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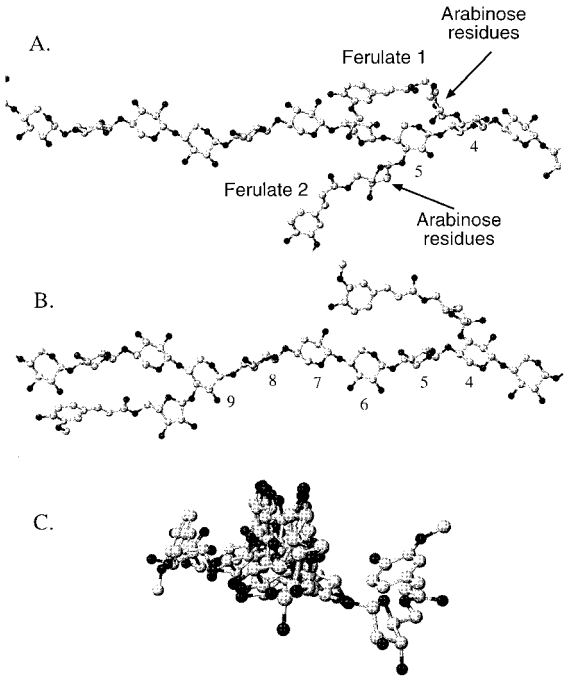

Scheme 1. Arabinoxylan models used to test feasibility of intramolecular diferulate formation. A, Feruloylated arabinosyl units on adjacent xylose residues of the xylan backbone (Table 1 FXXF). For all other positions the one feruloylated arabinosyl unit (left hand side or reducing end of the xylan) was held on xylose four while the second feruloyated arabinosyl unit was moved down the chain one xylose unit at a time (not all xylose units are shown in the schematic). B, Feruloylated arabinosyl unit one is on xylose four of the reducing end and feruloylated arabinosyl unit two is on xylose nine (Table 1 FXXXXXXF). C, An end view of feruloylated arabinoxylan showing the extended ribbon conformation with ferulates extending out and parallel to the xylan backbone.

energy conformation had been successfully obtained. For cross-linking experiments, the two feruloylated arabinosyl units attached to the xylan backbone were coupled to form one of the possible intramolecular diferulate molecules (Fig 1). The newly formed structures (xylan backbone with intramolecular diferulates) were optimised as before. A second set of experiments was conducted in which the xylan backbone was held rigid to maintain the extended ribbon conformation. All minimised structures were pushed out of the low energy state and reoptimised to ensure the lowest energy conformation had been reached.

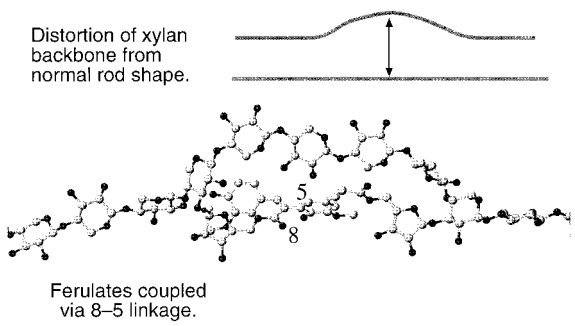

Figure 1. Minimised structure of feruloylated arabinoxylan when ferulates were separated by four xylose units and coupled by an 8–5 linkage. When the xylan backbone was allowed to relax minimised structures resulted in a distortion of the ribbon conformation. This type of distortion was evident for most of the coupling reactions when the xylan backbone was allowed to freely relax.

RESULTS AND DISCUSSION

Initial ferulated arabinoxylan molecules used to test the feasibility of intramolecular cross-linking are represented in Scheme 1. Ferulated arabinosyl components were attached to the xylan backbone with increasing numbers of xylose residues between the attachment xylose residues, starting with ferulates on adjacent xyloses. When ferulates were left uncoupled, minimised structures always resulted in an inflexible extended ribbon conformation with a three-fold screw axis. All ferulates reached a final position parallel to the main xylan axis (Scheme 1C). These minimised structures corresponded quite well to experimental data.[8] This would indicate that the molecular modelling program predicts conformational characteristics of ferulated arabinoxylan structures.

In the first set of coupling experiments, the xylan backbone was allowed to freely relax in order to reach the lowest possible energy state. Energy values before coupling were compared to the lowest possible values obtained as a result of coupling the ferulates to form the dehydrodiferulates typically found in plant walls. When the xylan backbone can relax, almost all diferulate configurations resulted in optimised molecules with energy values within acceptable ranges ($20 \pm 5\,\mathrm{kcal\,mol^{-1}}$ is considered the minimum energy barrier at room temperature[9]). Inspection of the optimised molecular structures, however, indicated distortion of the xylan backbone (see example in Fig 1) for almost every coupling scenario, even when ferulates were close enough to overlap. It appears that forcing the ferulates to covalently cross-link followed by optimisation of molecular conformation prefers to produce structures that change the configuration of the xylan backbone as opposed to making severe adjustments in the ferulate conformations. Based on experimental evidence it does not seem likely that the xylan would be sufficiently flexible, especially once it has been incorporated into the wall matrix.

In the second series of experiments, when the xylan backbone was held rigid to allow minimum flexing, fewer coupling reactions resulted in stable low energy molecules (Table 1). Distances of more than three xyloses could not produce stable low energy conformations for the coupled ferulated arabinoxylans. There was one exception, however. When the ferulates were separated by two xylose residues (ie ferulate one on xylose four and ferulate two is on xylose seven, Table 1 FXXXXF) the configuration for 5–5 diferulate formation resulted in a low energy molecule. In only this case was a stable molecule generated, possibly due to the correct

Table 1. Energy values in kcal for optimised molecular structures of ferulated arabinoxylan with the ferulated arabinosyl units positioned at various locations along the xylan backbone. Ferulates were either uncoupled or covalently linked to form intramolecular dehydrodiferulates. For this set of experiments the xylan backbone was in a locked conformation during minimization runs to maintain an extended infelxible ribbon conformation. The feasibility of specific cross-coupling reactions was determined by a comparison of the final optimum energy value for the uncoupled molecule with the energy of the appropriate ferulate dimer (comparisons are always within rows and an energy value of 20 ± 5 kcal mol^{-1} was used as the maximum energy difference for allowable conformations)

Ferulated arabinosyl units position on xylan backbone	*kcal mol^{-1} for optimised molecular conformation*				
	Type of covalent linkages between the two ferulates				
	Uncoupled	*8–O–4*	*8–8*	*5–5*	*8–5*
F F XX	244	*308*	*277*	259	*300*
F F XXX	216	*341*	*278*	*252*	*248*
F F XXXX	220	*254*	*271*	244	*255*
F F XXXXX	223	*336*	*862*	*259*	*403*
F F XXXXXX	220	*378*	—	*331*	—

Values in italic indicate minimisation energies that are too high for a stable coupling reaction to occur between two ferulates to produce the indicated diferulate molecule

spatial distance for ferulate rotation and 5–5 bond formation. In the case of the inflexible xylan backbone a few intramolecular diferulates were possible based on the optimized conformations (Table 1). The resulting molecular configurations required the ferulates to take conformations that were greatly different from their minimised structures obtained from unattached conformations. This would require the spontaneous adjustment of several dihedral angles forming the feruloylated arabinosyl unit.

To test the feasibility of spontaneous realignment of feruloylated arabinosyl units to conformations that would allow coupling, energy maps were constructed involving the major dihedral angles required to produce the low energy conformation of two of the diferulate molecules, 5–5 and 8–8 coupled on adjacent xyloses (Scheme 1A). In the case of the 8–8 coupled diferulate, the energy maps indicated that movement could occur to possibly allow the formation of this intramolecular diferulate, although the final energy is probably outside the acceptable range for a stable conformation (Table 1). For the 5–5 coupled configuration, energy barriers prohibited the complete adjustment of all the necessary dihedral angles to achieve the final low energy conformation, even though a low energy state could be produced when formation was forced.

CONCLUSIONS

Although intramolecular diferulates could be produced, their natural occurrence would seem to have a minimal probability based on the modelling experiments conducted. In most cases the formation requires either a relaxation of the xylan backbone or adjustment of the ferulated arabinosyl component through less energy stable conformations before arriving at the final low energy molecular conformation. The one exception was the formation of the 5–5 diferulates that seemed to be feasible when the individual ferulates were on positions four and seven. In this position the ferulates are essentially on the same side of the xylan backbone and free rotation can occur about the glycosyl linkage between arabinose and the xylan backbone, bringing the ferulates into proper orientation for 5–5 coupling. This suggests restricted positioning of ferulates to allow 5–5 coupling.

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